DYES METABOLIZED TO BENZIDINE (BENZIDINE DYE CLASS)*

First listed as a class in the *Ninth Report on Carcinogens*

CARCINOGENICITY

Benzidine-based dyes that are metabolized to benzidine are *known to be human carcinogens* based on the fact that 1) benzidine is a known human carcinogen (IARC V.1, 1972; IARC S.1, 1979; IARC V.29, 1982; IARC S.7, 1987; NTP, 1998), 2) metabolism of benzidine-based dyes to release free benzidine is a generalized phenomenon in humans and all experimental animal species studied, (Rinde and Troll, 1975; Lynn et al., 1980; Nony et al., 1980; Lowry et al., 1980; Martin and Kennelly, 1985), and 3) benzidine exposure from exposure to benzidine-based dyes is equivalent to exposure to equimolar doses of benzidine (Lynn et al., 1980).

The evidence that benzidine-based dyes that are metabolized to benzidine are human carcinogens is supported by experimental animal studies which have shown that all benzidine-based dyes that have been tested in experimental animals are animal carcinogens and therefore represent a carcinogenic risk to humans (NCI 108, 1978; IARC V.29, 1982; IARC S.4, 1982).

ADDITIONAL INFORMATION RELEVANT TO CARCINOGENESIS OR POSSIBLE MECHANISMS OF CARCINOGENESIS

Benzidine was one of the first chemicals for which an association of occupational exposure and increased cancer was recognized for humans. Increased incidences of urinary bladder cancer in humans were concluded to result from industrial exposure by the International Labor Office in 1921 (International Labour Office, 1921; cited by IARC V.29, 1982). Since that time, several IARC and NTP committees (IARC V.1, 1972; IARC S.1, 1979; IARC V.29, 1982; IARC S.7, 1987; NTP, 1998) have concluded that benzidine and its salts are carcinogens in numerous species including rats, mice, hamsters, dogs, and humans. The primary target organs for carcinogenicity induced by benzidine vary with species. Rats, mice, and hamsters develop increased incidences of hepatocellular carcinomas, mammary carcinomas in female rats, and Zymbal gland tumors in both sexes of rats. Dogs and humans develop increased incidences of urinary bladder cancer.

The first dyes based on the benzidine molecule were synthesized more than 100 years ago. Since a wide spectrum of colors could be achieved by varying the molecules' chromophores, linked to benzidine by an azo linkage (-N=N-), this facile and productive synthesis resulted in many excellent dyes. The variety of dyes based on benzidine is exemplified by the fact that 258 benzidine-based dyes were listed in the third edition of the Colour Index (Martin and Kennelly, 1985). Each of these dyes was formed by diazotization of benzidine with nitrous acid and then coupling the resulting diazonium salt with various chromophores to form compounds with azo linkages. Similar or different chromophores may be linked at each amino group of the benzidine molecule to form various bisazobiphenyl dyes. However, regardless of the chromophore(s) involved, the azo linkages of all benzidine-based dyes are essentially

* There is no CAS registry number assigned to dyes metabolized to benzidine.

chemically equivalent.

Just as the azo linkages between benzidine and chromophores are easily formed chemically, they are also easily broken by chemical or enzymatic reduction. Products of reductive cleavage of the dyes are free benzidine and the respective chromophores. One of the first reports of reductive cleavage of a benzidine-based dye in a biological system was that of Rinde and Troll (1975). That report indicated that each of four benzidine-based dyes was reduced to benzidine by primates, most probably by gastrointestinal bacteria. Later reports provided evidence that benzidine-based dyes are metabolized to free benzidine by humans (Lowry et al., 1980) and also rats and dogs (Lynn et al., 1980), and hamsters (Nony et al., 1980). Lowry et al. (1980) concluded that the amount of benzidine and its metabolites detected in urine of exposed workers could not have been accounted for by the minute amounts of free benzidine in the dyes to which they were exposed. Thus, evidence was provided to indicate that humans also metabolize benzidine-based dyes to free benzidine. The conclusion to be drawn from this series of studies is that reduction of benzidine dyes to release benzidine was a generalized phenomenon that occurred in most, if not all, species. By determining the quantities of benzidine and its metabolites excreted following administration of free benzidine versus three benzidine-based dyes, Lynn et al. (1980) also provided quantitative data for the reduction of benzidine-based dyes to free benzidine. Results of that study indicated evidence that each of the dyes studied was reduced to an amount of free benzidine equal to that observed from an equimolar dose of benzidine. Thus, the first evidence was provided to indicate that ingestion of benzidine-based dyes was equivalent to exposure to an equimolar dose of free benzidine.

Since occupational exposure to benzidine-based dyes has been most frequently associated with co-exposure to benzidine, it has been difficult to clearly establish their carcinogenicity in humans. Two recent studies have endeavored to address this problem by studying Chinese workers who remained in the same jobs for many years. Results of these studies were mixed. Whereas You et al. (1990) observed no increased incidence of tumors in workers exposed almost exclusively to benzidine-based dyes, Bi et al. (1992) reported that cancer incidences were elevated for workers exposed to both benzidine and benzidine-based dyes. Unfortunately, neither report was able to adequately document levels of exposure to either benzidine or the dyes. Evidence for the carcinogenicity of benzidine-based dyes in laboratory animals has been provided by studies in which three dyes, Direct Blue 6, Direct Black 38, and Direct Brown 95, were positive liver carcinogens in rats following an exposure of only 13 weeks (NCI 108, 1978; IARC V. 29, 1982; IARC S.4, 1982). The IARC evaluation of these results and benzidine-based dyes in general reached the following conclusion. "Although the epidemiological data were inadequate to evaluate the carcinogenicity to man of individual benzidine dyes, they, together with the presence of benzidine in the urine of exposed workers, provide *sufficient evidence* that occupational exposure to benzidine-based dyes represents a carcinogenic risk to man."

Profiles for individual dyes metabolized to benzidine that had been previously listed in the *Report on Carcinogens* as *reasonably anticipated to be human carcinogens* and are now listed as *known to be human carcinogens* follow this profile and include the following:

- Direct Black 38, CASRN. 1937-37-7, pp 76
- Direct Blue 6, CASRN. 2602-26-2, pp 79

PROPERTIES

Benzidine can be found as white or slightly reddish crystals or powder. It’s density is

1.250 at 20°C/4°C. It is slightly soluble in hot water, boiling ethanaol and diethyl ether. The various dyes that metabolize to benzidine have varying colors; from blue, to red, orange, brown and black.

USE

Dyes that metabolize to benzidine are mainly used to color textiles, rubber, plastic products, printing inks, paints, lacquers, leathers, and paper product. Approximately 50% of the dyes are applied to textiles, 45% to paper, and 5% to leather (NCI DCCR, 1975). While benzidine use has fallen dramatically in recent years due to its potential carcinogenicity, dyes that metabolize to benzidine are still used.

PRODUCTION

There are over 22 dyes that metabolize to benzidine that have been produced in the US. They are marketed under several hundred tradenames (NIOSH, 1983). In 1978 1-2 million lb of dyes that metabolize to benzidine were produces of imported (NIOSH 1980). U.S. sales and imports of dyes that metabolize to benzidine fluctuated between 1975-1978. In 1975 U.S. sales were 4.2×10^6 lb, while imports were 0.9×10^6 lb. Sales peaked in 1976 (6.6×10^6 lb) but steadily declined in 1977 (4.6×10^6 lb) and 1978 (1.9×10^6 lb). Imports slowly rose, with 0.6×10^6 lb being imported in 1976 to 1.3×10^6 lb in 1977, to 1.6×10^6 lb in 1978 (U.S. EPA, 1980).

EXPOSURE

The primary routes of potential human exposure to dyes that metabolize to benzidine are inhalation, ingestion, and dermal contact. These dyes may enter the respiratory tract from accidental releases into the air; into the gastrointestinal tract from contaminated fingers, cigarettes, or food; and onto the skin directly or from contaminated clothing and gloves. While most of the dyes that metabolize to benzidine are only permitted to be used in closed systems, accidental releases of these dyes could lead to occupational and environmental exposure.

In most cases, dyes that metabolize to benzidine are hazards only in the vicinity of dye and pigment plants where wastes may escape or be discharged. The National Occupational Exposure Survey (1981-1983) estimated that 28,442 workers were potentially exposed to dyes that metabolize to benzidine (NIOSH, 1984). The National Occupational Hazard Survey (1972-1974) estimated thatn 20,470 workers were potentially exposed to dyes that metabolize to benzidine (NIOSH, 1976).

REGULATIONS

In 1980, the CPSC collected economic and toxicological data to propose a ban on the use of benzidine-based dyes in direct consumer dye products. CSPC also completes studies on the dermal penetration of two benzidine congener dyes with negative results. The use of benzidine congener dyes in consumer products and commercial textile applications has been decreased voluntarily. Therefore, CSPC voted to deny the petition that requested a ban of these consumer dye products. Educational material have been developed to warn artists of the potential hazard of these dyes.

EPA regulates benzidine under the Clean Water Act (CWA), the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), the Resource Conservation and Recovery Act (RCRA), the Superfund Amendments and Reauthorization Act (SARA), and the Toxic Substances Control Act (TSCA). Effluent discharge guidelines have been set under CWA, and benzidine is subject to reporting rules under CWA, SARA, and TSCA. A reportable quantity (RQ) of 1 lb (0.454 kg) has been proposed for benzidine under CERCLA. It is regulated as a hazardous constituent of waste under RCRA. FDA, under the Food, Drug, and Cosmetic Act (FD&CA), also regulates the amount of benzidine in various color additives for use in food, drugs, and cosmetics. The benzidine concentration in food colorants is limited to 1 ppb, except for D&C Red #. 33, which can contain up to 20 ppb benzidine. NIOSH (1994) has recommended that exposure to benzidine be the lowest feasible concentration. OSHA, which has established protective standards for occupational exposure to benzidine, regulates benzidine under the Hazard Communication Standard and as a chemical hazard in laboratories. Regulations are summarized in Volume II, Table A-22.